Methylome Analysis

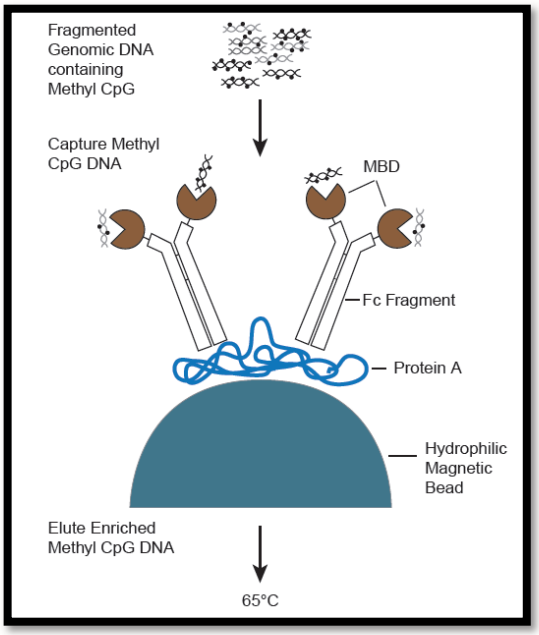
Targets

DNA methylation is a biochemical process fundamental for the physiological development of the organism and for the cell differentiation in the superior organisms. In adult tissues the methylation is concentrated on the CpG dinucleotides, collected together in *CpG islands* at the 5' of regulatory regions, *CpG shores* (placed at <2 Kb from the islands), and *CpG shelves* (at <2 kb from the *shores*). The distribution of methylated cytosines in the genome has a role in the epigenetic control of gene expression and alterations in DNA methylation levels (iper- or ipo-methylation) are involved in different processes at the basis of cancer, cellular senescence, genetic diseases, aging.

Procedure

The enrichment step of methylated regions can be optimized and performed in Genomnia or it can be set up and realized in the customer's laboratory. In this second option we want to highlight that, as requested from the Ion Torrent library construction protocol, it is necessary that the DNA enriched for methylated regions is double stranded (dsDNA). Genomnia personnel is available for eventual technical-scientific suggestions aimed at satisfying this requisite.

The adopted strategy is defined as *methyl-CpG binding domain* (MBD) and allows to concentrate the sequencing only on the genomic regions involved in the methylation process. The following picture gives a graphical representation of what described below.



In general, the *MBD pull-down* method exploits the functional properties of the MBD domain of many proteins being part of chromatin complexes to capture DNA regions in which the CpG dinucleotide cytosines are methylated.

As shown in the picture, in the system that we exploited the MBD domain of the human protein MBD2 is fused to the Fc tail of the human IgG1. The latter interacts with the A protein monomers present on the surface of hydrophilic paramagnetic beads. Since Fc fragment is a dimer and two dimeric Fc units can bind to a protein A monomer, four MBD domains for each protein A are available to interact with the methylated DNA fragments, thus increasing the binding affinity.

The genomic DNA is initially reduced in 50-500 bp fragments (with the majority of molecules having a size of around 200 bp), and in a second step the CpG methylated regions are selectively captured thanks to the previously described procedure. The double-stranded enriched DNA is eluted through incubation at 65°C and used as a template to produce a fragment library. The sequencing is performed on Ion Torrent S5 platform, with 200 bp reads and a sequencing depth of about 20 million of sequences for sample.

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Necessary sample quantity

The following table summarizes the DNA amount required to perform MBD-seq analysis starting from total genomic DNA or from DNA samples already subjected to the enrichment procedure. If the available DNA amount less than the lower values indicated, Genomnia will be glad to evaluate with customers the feasibility of the experiment, collaborating for the optimization of experimental conditions.

Starting material	Quantity (ng)
Genomic DNA	500 - 5000
DNA enriched in methylated regions (MBD)	10 - 100

Bioinformatic Analysis

The bioinformatic analysis of methylation experiments is available in two different levels.

In the level I bioinformatic analysis [MBD-BF01], we align the sequences to the reference genome and we elaborate the mapping statistics. We generate quality metrics and enrichment diagnostics in textual and graphical format and we identify through a proprietary procedure based on the Bioconductor MEDIPS package the genomic methylated regions.

In the second level bioinformatic analysis [MBD-BF02] we identify differentially methylated regions in agreement with the comparisons associated with the project. We then execute an annotation at the level of gene structure of the differentially methylated regions, followed by the functional analysis of differentially methylated genes.

In the third level bioinformatic analysis [MBD-BF03], we execute all the analyses described in MBD-BF01. In addition, we estimate the methylation of trasponible sequences at elevated redundancy (LINE, SINE) and we conduct a methylation differential analysis in the repeat families following the comparisons associated with the project.

Ordering informations

Product	Catalogue N.
QC: Control of quality and size of DNA MBD/CHIP enriched	DNA05
QC: quality control of DNA preparations	DNA06
Enrichment of MBD from genomic DNA	MBD
Preparation of DNA library with barcodes	LDb
Forward sequencing 200 bp tags with barcode	SEQI200B
Bioinformatic Analysis I: Methylation (identification of methylated regions)	MBD-BF01
Bioinformatic Analysis II: Methylation (differential analysis of methylated regions)	MBD-BF02
Bioinformatic Analysis III: Methylation (SINE/LINE differential analysis)	MBD-BF03

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